[JP,2002-058483,A]

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FULL CONTENTS CLAIM + DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART TECHNICAL PROBLEM MEANS EXAMPLE

[Translation done.]

EXAMPLE

[Example] Hereafter, the example of an examination is given and this invention is explained in detail.

[0041]

[The example 1 of an examination] The real-time step RT-PCR method adopted by the creation exam of the calibration curve by the real-time detecting method carries out simultaneously the reaction from which a maximum of 96 differed using 96 wells, and can measure a maximum of 96 specimen at once. Moreover, RT reaction and a PCR reaction can be performed within 1 tube.

[0042] The principle of this quantum uses FRET produced when a wavelength field overlaps between the fluorescence wavelength of one pigment (reporter pigment), and the excitation light wavelength of the pigment (quencher pigment) of another side using two kinds of adjoining fluorochromes. Hybridization is carried out to cDNA of the specific glutathione-S-transferase origin in which the probe (TaqMan probe) combined with both ends amplified two sorts of fluorochromes which produce FRET by PCR. The extension reaction of PCR starts in this status, and it is Taq. A TaqMan probe is hydrolyzed by 5'-3' endonuclease activity which a DNA polymerase has. Since a reporter pigment ****s, the physical distance between quencher pigments arises, the fluorescence intensity of the reporter pigment controlled by FRET increases and the increase in this fluorescence intensity is proportional to the increase of stock of the amplification product of PCR By measuring this for every PCR reaction, a desired quantum becomes possible.

[0043] In the exam, FAM was used as a reporter pigment and TAMRA was used for the three-dash terminal side as a quencher pigment at the five prime end side which is a probe. This etc. performed combination of each pigment, and creation of the TaqMan probe by this according to the procedure (Genome Res., 6 (10), and 986 (1996)) given in literature.

[0044] Moreover, the oligonucleotide as each primer and a probe was compounded using the substrate (dNTP) and the regular reagent using DNA / RNA-biosynthesis machine made from ABI as an automatic synthesizer.

[0045] As a specimen RNA, all the RNA refined from adult's brain, adult's kidney, adult's lungs, adult's small intestine, embryonic liver, and the adult's liver Poole was used. In addition, each of all the RNA, such as this, was purchased from Clonetec (Clontech

Laboratories, Inc.).
[0046] All the RNA refined from adult's brain, adult's kidney, adult's lungs, adult's small intestine, embryonic liver, and the adult's liver Poole was diluted with a RNase freelancer's water, and was made into 20microg/mL. Then, an equivalent amount of these six kinds were mixed every, and it carried out to calibration curve creation. Henceforth, it diluted with the common ratio 5 times using the yeast tRNA of 50microg/mL (Yeast tRNA, product made by GIBCO). 5microL was used for measurement.
[0047] RT-PCR reactions are a 300nM forward primer, a 900nM reverse primer, and a 200nM TaqMan probe. Using included TaqMan One-Step RT-PCR Master Mix Reagents Kit (PE Applied Biosystems), [the system of 50microL / tube] ABI PRISMTM7700 It carried out in Sequence Detection System (PE Applied Biosystems).
[0048] After it kept it warm in 30 minutes at 48 degrees C and temperature conditions kept them warm in 10 minutes at 95 degrees C, they were performed for 15 seconds at 95 degrees C, performed the cycle for 1 minute 50 times at 60 degrees C, and measured fluorescence intensity for every cycle.
[0049] The result (calibration curve) of the above-mentioned examination done using the primer pair of arrangement and probe which are shown in each arrangement number to each above mentioned enzyme is shown in Table 1.
[0050]
[Table 1]

酵素	検量線		相関係数	定量限界 (反応液量；50 μL)
	傾き	切片	r	(pg 全RNA)
(1) GSTP1	-3.40	38.20	1.00	1.28
(2) GSTT1	-3.21	37.78	1.00	6.4
(3) GSTT2	-4.01	43.15	0.99	32
(4) GSTM1b	-3.80	45.61	0.99	32
(5) GSTM2	-3.84	45.19	1.00	160
(6) GSTM3	-3.31	39.59	1.00	32
(7) GSTM4	-3.55	39.63	1.00	32
(8) GSTM5	-4.31	47.95	1.00	800
(9) GSTA1-1	-3.68	42.52	1.00	4000
(10) GSTA2	-4.65	46.29	1.00	800
(11) GSTA3	-6.38	62.04	0.99	4000
(12) GSTA4	-3.31	39.94	1.00	6.4
(13) MGST1	-3.29	36.29	1.00	6.4
(14) MGST2	-3.66	41.41	1.00	32
(15) MGST3	-3.33	37.03	1.00	32
(16) MGST111	-2.88	41.54	1.00	32
(17) GSTZ 1	-3.27	38.95	1.00	6.4

[0051] When the calibration curve diluted with the common ratio 5 times from 100000pg all RNA / the amount of 50microL reaction mixture was created from Table 1, it is about GSTP1, 1. It had a fixed quantity of sexes to 28pg all RNA / the amount of 50microL reaction mixture, and the coefficient of correlation (r) of the calibration curve which made the quantitation limit 4000pg all RNA from 1.28pg about other enzyme was 0.99 or more.
[0052]
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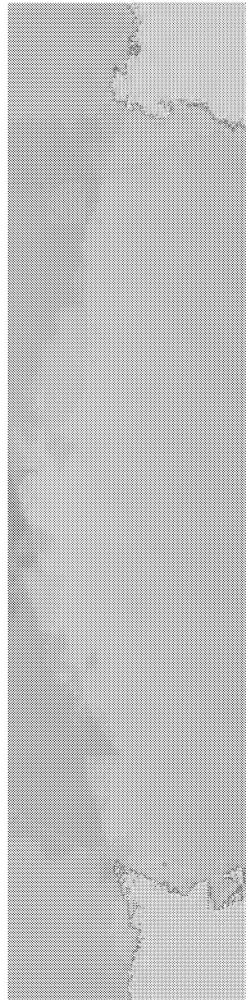

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